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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
G67773 RS/gf		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/04079	14/06/1999	23/06/1998
International Patent Classification (IPC) or national classification and IPC		
C12N15/10		
Applicant		
BIOSEARH ITALIA SPA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/12/1999	08.09.00
Name and mailing address of the international preliminary examining authority:	Authorized officer
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/04079

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-89 as originally filed

Claims, No.:

1-35 as received on 14/06/2000 with letter of 14/06/2000

Drawings, sheets:

1/11-11/11 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 18-22,24-26,28-30.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☒ the claims, or said claims Nos. 18-22,24-26,28-30 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5-8,12-17,23,27,31
	No:	Claims	1-4,9-11,32-35
Inventive step (IS)	Yes:	Claims	5,6,13-17,23,27,31
	No:	Claims	7,8,12
Industrial applicability (IA)	Yes:	Claims	1-17,23,27,31-35
	No:	Claims	

2. Citations and explanations

see separate sheet

Ad item III, IV and V:

A method for transferring the production of a "natural product" from an actinomycete donor (i.e. Streptomyces aureofaciens) to a "different" actinomycete host (i.e. Streptomyces lividans) by using an "E. coli-Streptomyces Artificial Chromosome" that carries a gene cluster governing the biosynthesis of said natural product (i.e. tetracycline) is described in D1 (EP-A-0468220).

The alleged difference between the prior art is the fact that the plasmids used in the present application are suitable for the cloning and transfer of "larger" fragment.
The expressions "large" or "larger", however, are not suitable to characterise the claimed method or products and/or to distinguish them from the prior art.

The **key features** of the present application are the two plasmids which allow the cloning of "large" fragments, i.e. the plasmids "pPAC-S1" and "pPAC-S2".

Thus, a basis for acceptable set of claims can be found in those claims wherein said plasmids are referred to, provided the arbitrary designations are replaced by a more suitable characterisation (see e.g. the method Claim 5 and product claims which contain said plasmids; see e.g. Claims 13 to 17).

However, most of the other claims fail to indicate the essential feature of the present application. Thus, claims which merely relate to an "E. coli-Streptomyces Artificial Chromosome" and "large" fragments are not distinguishable from the cosmid vectors of D1 (see e.g. Claim 11) or not novel and/or inventive over the method described in D1 (see Claims 1 to 4, 7, 8 and 32-35))

As far as the resulting products of the processes are concerned, they are objectionable for several reasons.

In their broadest definition they are not distinguishable from D1 (see Claims 9 and 10).
Insofar as they relate to an "actinomycete host" which is constructed by the transfer of a specific cluster (see Claims 18-22, 24-26 and 28-30),

(a) they lack an inventive activity, since the transfer of clusters is also possible by the methods of the art i.e. the transfer of clones containing only parts of the clusters

(b) all of these claims are not sufficiently supported by the description, since the corresponding clusters have not even been isolated

(c) the different hosts containing different clusters are not linked by a common inventive concept, since the inventive feature i.e. the plasmids referred to above are no longer present in the hosts.

CLAIMS

We claim:

1) A method for transferring the production of a natural product from an actinomycete donor organism that is the original producer of said natural product to a different actinomycete host, where this transfer is achieved by means of an *E. coli-Streptomyces* Artificial Chromosome that carries a gene cluster governing the biosynthesis of said natural product derived from said donor organism characterized in that it comprises the steps of:

(a) isolating large fragments of chromosomal DNA of the actinomycete donor organism of a size which encompasses the gene cluster that directs the biosynthesis of the natural product;

(b) constructing a suitable vector capable of accomodating said large fragments of chromosomal DNA and of introducing and stably maintaining said large fragments of DNA into an *E. coli* host;

(c) constructing an *E. coli-Streptomyces* Artificial Chromosome by inserting said large fragments of chromosomal DNA of step (a) into the above said vector of step (b) and selecting the *E. coli-Streptomyces* Artificial Chromosome comprising the entire gene cluster construct that directs the biosynthesis of the above said natural product;

(d) transforming an actinomycete host different from the donor actinomycete host with *the E. coli-Streptomyces* Artificial Chromosome of step (c) that carries the gene cluster governing the biosynthesis of said natural product wherein the actinomycete host carries a region which is specific for the integration of the *E. coli-Streptomyces* Artificial Chromosome.

2) A process as in claim 1 wherein the large fragments of genomic DNA of the actinomycete donor organism of step (a) are obtained by partial digestion of the chromosomal DNA of said actinomycete donor organism.

3) A process as in claim 1 wherein the large fragments of the genomic DNA of step (a) are obtained by reconstruction through interplasmid homologous recombination from a set of pre-existing smaller segments of partially overlapping DNA cloned from the genome of the actinomycete donor organism, which set of segments encompass the entire gene cluster that directs the biosynthesis of said natural product.

4) A process as in claim 1, 2 or 3 wherein the suitable vector of step (b) contains an *int-attP* region, where the *int* insert preferably derives from phage ΦC31.

5) A process as in claim 4 wherein the suitable vector of stp (b) is the plasmid pPAC-S1 or pPAC-S2 (Fig. 2) further characterized by the following features:

a) ability to accomodate DNA inserts up to 300kb,
b) low copy number in *E. coli* for increased stability,
c) ease of propagation because of the inclusion of the pUC19 stuffer segment,
d) presence of BamHI, XbaI or ScaI cloning sites, with positive selection inserts for resistance to sucrose,
e) T7 and SP6 promoters flanking the cloning site,
f) resistance to kanamycin in *E. coli*,
g) resistance to thiostrepton and site specific integration at the ΦC 31 *attB* site in *Streptomyces* conferred by the *int-tsr* cassette,
h) pPAC-S1 carries the *int* gene of the *int-tsr* cassette adjacent to the *sac*B gene while pPAC-S2 carries the *tsr* gene of *tsr int-tsr* cassette adjacent to the *sac*B gene

6) A process as in claim 1 wherein the *E. coli-Streptomyces* Artificial Chromosome is the plasmid pPAC-S1 or pPAC-S2 according to claim 5 modified by insertion of the entire gene cluster that directs the biosynthesis of the natural product.

7) A process as in claim 4 wherein the integration of the *E. coli-Streptomyces* Artificial Chromosome into the actinomycete host occurs at the *attB* site carried by said actinomycete host and is mediated by the *int-attP* function specified by the *E. coli-Streptomyces* Artificial Chromosome

8) A process as in claim 1, 2, 3, 4, 5, 6 or 7 wherein the actinomycete host is a *Streptomyces lividans* strain.

9) An actinomycete production host that is constructed from an actinomycete host by transfer of a cluster from a donor organism according to claim 1.

10) An actinomycete production host as in claim 9 that is a *Streptomyces lividans* strain.

11) An *E. coli-Streptomyces* Artificial Chromosome that carries a gene cluster directing the biosynthesis of a natural product.

12) An *E. coli-Streptomyces* Artificial Chromosome of claim 11 that contains an *int-attP* region and a selection marker.

13) An *E. coli-Streptomyces* Artificial Chromosome of claim 12 that is the vector pPAC-S1 of claim 5 modified by insertion of a gene cluster directing the biosynthesis of a natural product.

14) An *E. coli-Streptomyces* Artificial Chromosome of claim 12 that is the vector pPAC-S2 of claim 5 modified by insertion of a gene cluster directing the biosynthesis of a natural product.

15) An *E. coli-Streptomyces* Artificial Chromosome as in claim 11 that is the construct PAD6, which is the vector pPAC-S1 of claim 5 modified by insertion of the gene cluster of *P.rosea* characterized in that:

a) it carries an insert of about 90-kb from the genome of *P.rosea*, where the left and right ends of such insert are delimited by the sequences SEQIDN. 9 and SEQIDN. 10, respectively, cloned into said vector pPAC-S1 of claim 5.

b) after digestion with *Eco*RI yields fragments of 47, 46, 8.1, 4.6, 2.2, 0.5 and 0.1 kb,

c) after digestion with *Dra*I yields fragments of 102, 4.2 and 0.6 kb.

16) An actinomycete production host as in claim 9 that carries the construct PAD6 of claim 15..

17) An actinomycete production host as in claim 16 that is a *Streptomyces lividans* strain.

18) An *E. coli-Streptomyces* Artificial Chromosome as in claim 11 that carries a gene cluster from *Planobispora rosea*

19) An actinomycete production host as in claim 9 that carries a gene cluster from *Planobispora rosea*.

20) An actinomycete production host as in claim 9 that contains the *E. coli-Streptomyces* Artificial Chromosome carrying the rapamycin gene cluster.

21) An actinomycete production host as in claim 20 that is a *Streptomyces lividans* strain.

22) An *E. coli-Streptomyces* Artificial Chromosome as in claim 11 that carries the rapamycin gene cluster.

23) An *E. coli Streptomyces* Artificial Chromosome as in claim 22 that is the vector pPAC-S1 or pPAC-S2 of claim 5 modified by insertion of the gene cluster directing the biosynthesis of rapamycin.

24) An actinomycete production host as in claim 9 that contains the *E. coli-Streptomyces* Artificial Chromosome carrying the erythromycin gene cluster.

25) An actinomycete production host as in claim 24 that is a *Streptomyces lividans* strain.

26) An *E. coli-Streptomyces* Artificial Chromosome as in claim 11 that carries the erythromycin gene cluster.

27) An *E. coli-Streptomyces* Artificial Chromosome as in claim 26 that is the vector pPAC-S1 or pPAC-S2 of claim 5 modified by insertion of the gene cluster directing the biosynthesis of erythromycin.

28) An actinomycete production host as in claim 9 that contains the *E. coli-Streptomyces* Artificial Chromosome that carries the rifamycin gene cluster.

29) An actinomycete production host as in claim 28 that is a *Streptomyces lividans* strain.

30) An *E. coli-Streptomyces* Artificial Chromosome as in claim 11 that carries the rifamycin gene cluster.

31) An *E. coli-Streptomyces* Artificial Chromosome as in claim 30 that is the vector pPAC-S1 or pPAC-S2 of claim 5 modified by insertion of the gene cluster that direct the biosynthesis of rifamycin.

32) A process for the production of a natural product by cultivating an actinomycete strain capable of producing said natural product in the presence of nutrient medium, isolating and purifying said natural product, characterized in that the actinomycete strain capable of producing said natural product is a an actinomycete production host obtained according to the method of claim 1.

33) A process as in claim 32 wherein the actinomycete production host is a *Streptomyces lividans* or *Streptomyces coelicolor* strain.

34) A process as in claim 32 wherein the production host is one of those described in any of claims 19, 20, 21, 24, 25, 28 or 29.

35) A process as in claim 32, for the production of a natural product selected from rapamycin, erythromycin and rifamycin.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
G67773 RS/mg		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 04079	14/06/1999	23/06/1998
Applicant		
BIOSEARH ITALIA SPA et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 3

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL RCH REPORT

International Application No
PCT/EP 99/04079

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/10 C12N15/52 C12N15/70 C12N15/76 C12N1/21
C12P19/62 C12P17/18 //(C12N1/21,C12R1:465)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 468 220 A (AMERICAN CYANAMID CO) 29 January 1992 (1992-01-29) examples 1-10 ---	1-5, 8-15,42, 43
Y	F. MALPARTIDA AND D.A. HOPWOOD: "Molecular cloning of the whole biosynthetic pathway of a Streptomyces antibiotic and its expression in a heterolohous host" NATURE, vol. 309, 31 May 1984 (1984-05-31), pages 462-464, XP002116074 MACMILLAN JOURNALS LTD., LONDON,UK cited in the application the whole document --- -/--	1-5, 8-15,42, 43

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 1999	05/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hornig, H

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 07868 A (CIBA GEIGY AG ;SCHUPP THOMAS (CH); TOUPET CHRISTIANE (FR); ENGEL N) 26 February 1998 (1998-02-26) page 14, line 27 -page 17, line 3 ---	1,10, 36-40
Y	J.S. TUAN ET AL.: "Cloning of genes involved in erythromycin biosynthesis from Saccharopolyspora erythraea using novel actinomycetes-Escherichia coli cosmid" GENE, vol. 90, no. 1, 31 May 1990 (1990-05-31), pages 21-29, XP002116075 ELSEVIER SCIENCE PUBLISHERS,B.V.,AMSTERDAM,NL; the whole document ---	1-5, 8-15, 30-34, 36-40, 42-45
Y	M. BIERMAN ET AL.: "Plasmid cloning vectors for the conjugal transfer of DNA from Escherichia coli to Streptomyces spp." GENE, vol. 116, no. 1, 1 July 1992 (1992-07-01), pages 43-49, XP002116076 ELSEVIER SCIENCE PUBLISHERS,B.V.,AMSTERDAM,NL; page 43, line 1 - line 12 Plasmid pOJ444 page 45, left-hand column, line 14 -page 46, left-hand column, line 3 ---	1-5, 8-15, 30-34, 36-40, 42-45
A	T. SMOKVINA ET AL.: "Construction of a series of pSAM2-based integrative vectors for use in actinomycetes" GENE, vol. 94, no. 1, 28 September 1990 (1990-09-28), pages 53-59, XP002116077 ELSEVIER SCIENCE PUBLISHERS,B.V.,AMSTERDAM,NL; the whole document ---	1-45
A	IOANNOU P A ET AL: "A NEW BACTERIOPHAGE P1-DERIVED VECTOR FOR THE PROPAGATION OF LARGE HUMAN DNA FRAGMENTS" NATURE GENETICS, vol. 6, 1 January 1994 (1994-01-01), pages 84-89, XP000770742 ISSN: 1061-4036 cited in the application the whole document --- -/--	1-45

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
A	SHIZUYA H ET AL: "CLONING AND STABLE MAINTENANCE OF 300-KILOBASE-PAIR FRAGMENTS OF HUMAN DNA IN ESCHERICHIA COLI USING AN F-FACTOR-BASED VECTOR" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 89, 1 September 1992 (1992-09-01), pages 8794-8797, XP000573603 ISSN: 0027-8424 cited in the application the whole document	1-45
A	SCHWECKE T ET AL: "The biosynthetic gene cluster for the polyketide immunosuppressant rapamycin" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,August 1995 (1995-08), pages 7839-7843, XP002079432 ISSN: 0027-8424 cited in the application the whole document	1-45
A	GAISSER S ET AL: "ANALYSIS OF SEVEN GENES FROM ERYAL-ERYK REGION OF THE ERYTHROMYCIN BIOSYNTHETIC GENE CLUSTER IN SACCHAROPOLYSPORA ERYTHRAEA" MOLECULAR AND GENERAL GENETICS, vol. 256, 1 October 1997 (1997-10-01), pages 239-251, XP002061261 ISSN: 0026-8925 the whole document	1-45

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter[illegible]nal Application No
PCT/EP 99/04079

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0468220 A	29-01-1992	AU 652766 B	08-09-1994
		AU 8134091 A	30-01-1992
		CA 2047833 A	27-01-1992
		JP 4346786 A	02-12-1992
		PT 98428 A,B	30-06-1992
		SG 43237 A	17-10-1997
		US 5589385 A	31-12-1996
		US 5866410 A	02-02-1999
WO 9807868 A	26-02-1998	AU 4119597 A	06-03-1998
		EP 0929681 A	21-07-1999